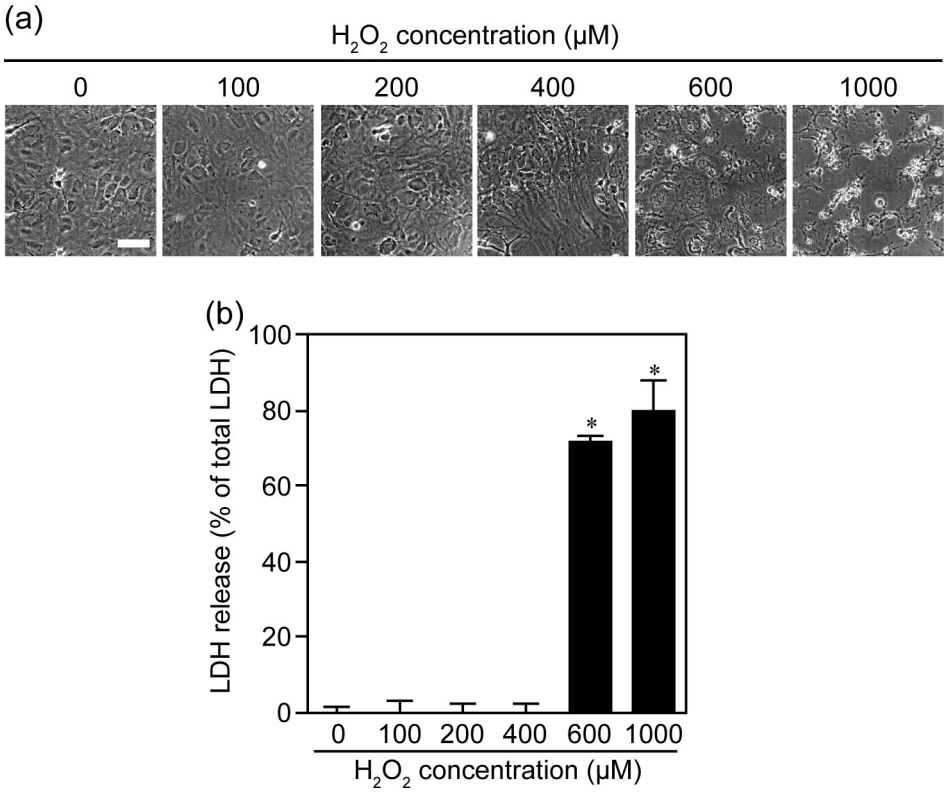
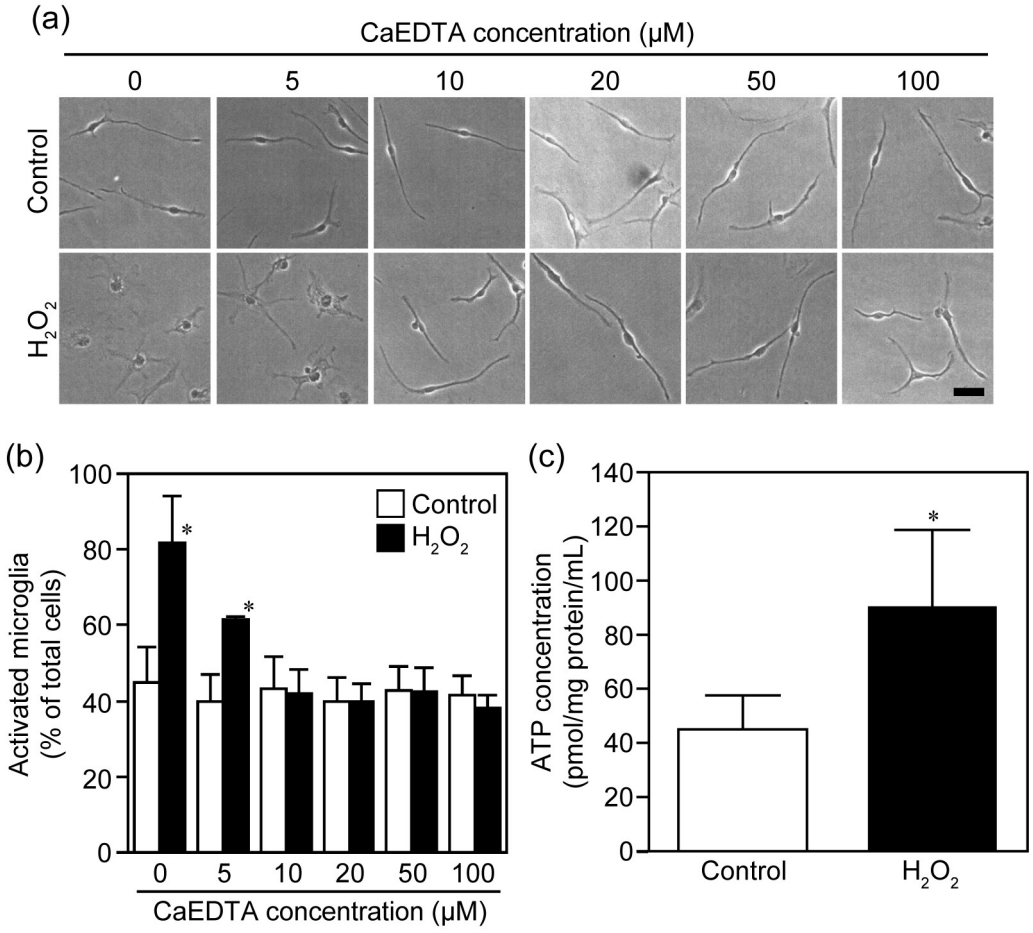
Electronic Supplementary Material (ESI) for Metallomics.



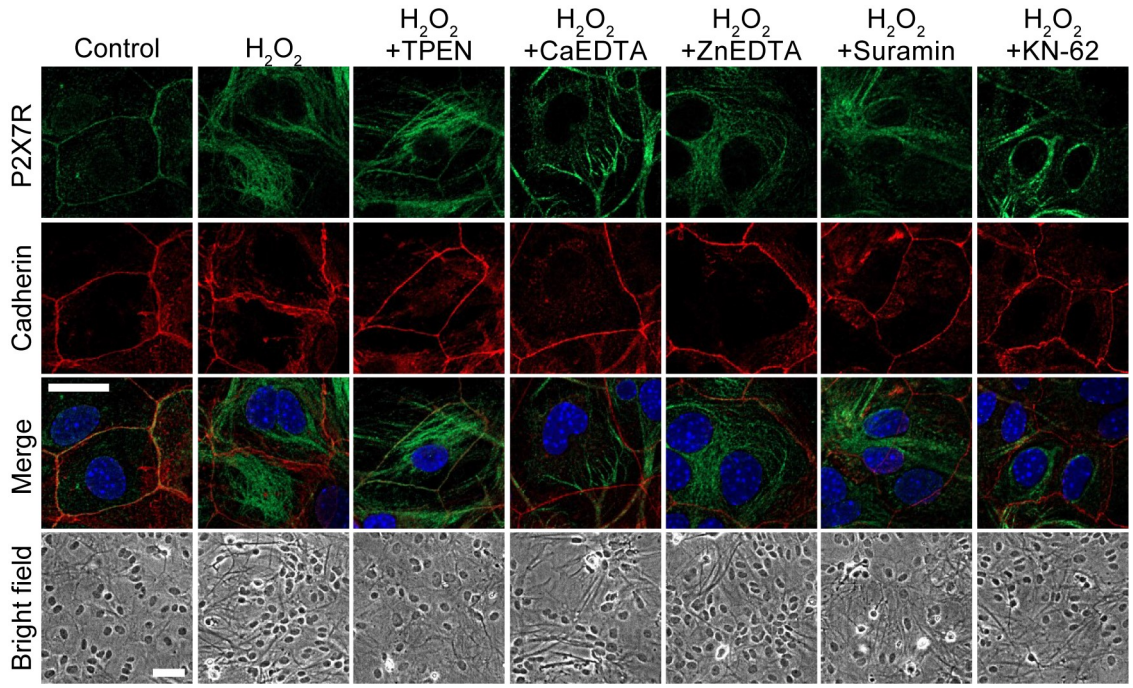
sFigure 1. Effect of H_2O_2 treatment on astrocytic viability.

After 10-min pre-incubation in BSS, astrocytes were treated with the indicated concentrations of H_2O_2 for 24 h at 37°C in a CO_2 incubator. Thereafter, the LDH concentrations in the medium were measured. Representative photomicrographs for three independent experiments are shown in panel a. In panel b for quantitative results for LDH release, each column represents the mean±SD (N=3). Bar = 40 μm.



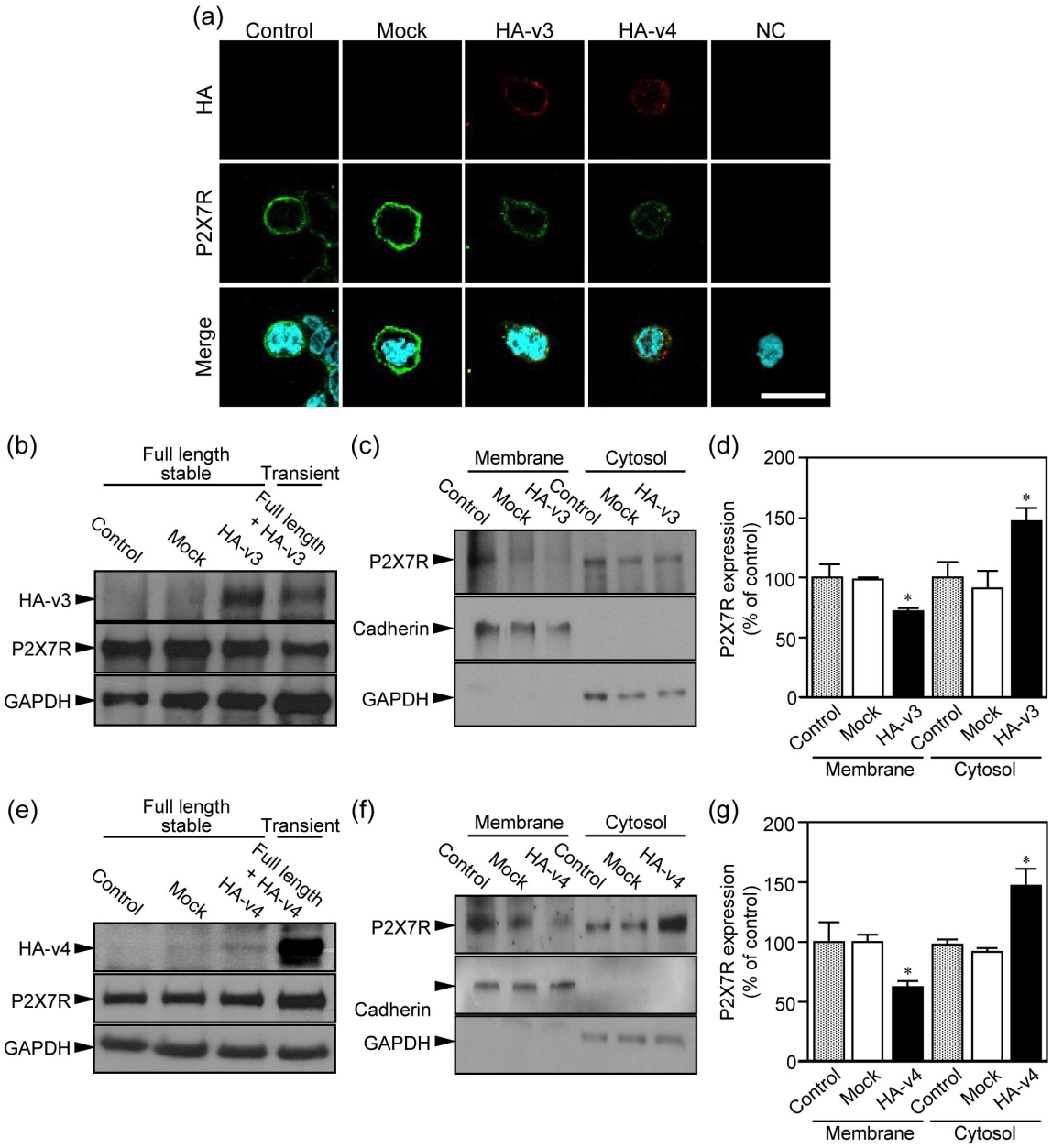
sFigure 2. H_2O_2-treated astrocytes release zinc and ATP.

(a, b) After 10-min pre-incubation in BSS, astrocytes were incubated with 400 μM H_2O_2 in the presence or absence of 2 μM TPEN for 24 h in a CO_2 incubator, and then the media were collected. After heat inactivation (95°C for 5 min) and centrifugation of the media, they were administered to microglial cultures, and then the cells were incubated for 2 h in the presence or absence of the indicated concentrations of CaEDTA in a CO_2 incubator. Thereafter, the morphological change of microglia was evaluated. Representative photomicrographs and the quantitative results for three independent experiments are shown in panels a and b, respectively. Each column represents the mean±SD (N=3). *p<0.05 (*vs*. respective control). Bar = 40 μm. (c) After 10-min pre-incubation in BSS, astrocytes were incubated with 400 μM H_2O_2 for 2 h in a CO_2 incubator, and then the media were collected. After heat inactivation (95°C for 5 min) and centrifugation of the media, ATP concentrations in them were measured. Each column represents the mean±SD (N=5). *p<0.05 (*vs*. respective control).



sFigure 3. Extracellularly released zinc and ATP are not involved in translocation of P2X7R in oxidative stress-loaded astrocytes.

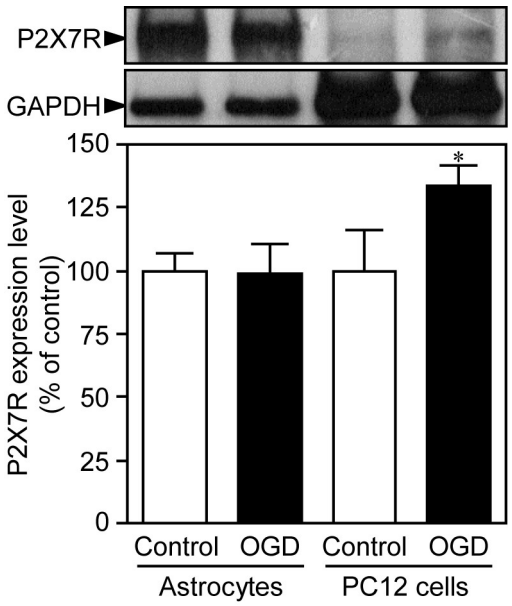
(a) After 10-min pre-incubation in BSS, astrocytes were incubated with 400 μM H_2O_2 in the presence and absence of 2 μM TPEN, 50 μM CaEDTA or ZnEDTA (a negative control for CaEDTA), 50 μM suramin, or 10 μM KN-62 for 24 h in a CO_2 incubator. Thereafter, immunostaining for P2X7R (green) with antibodies against the P2X7R-C-terminal domain and cadherin (red) as a marker for plasma membranes was performed, with counterstaining of nuclei with 2 μg/mL of Hoechst33258 (blue). Although the data are not shown, none of the chelators/antagonists alone had any effect on the expression profile of P2X7R in astrocytes, and a negative control for immunostaining, which was performed by omitting the 1st antibodies, did not give any signals. Representative photomicrographs for three independent experiments are shown. Bar = 40 μm.



sFigure 4. Expression of P2X7R-v3 or -v4 induces translocation of P2X7R from the plasma membrane to the cytosol.

HEK293T/mP2X7R cells were transfected with cDNA for HA-v3 or -v4, respectively, followed by 48-h post-incubation in 10% FBS-EMEM, and then their expression levels were determined by immunocytochemistry (a) and Western blotting (b, c, e, f). As a positive control for expression of P2X7R-v3 and -v4, HEK293T cells transfected with cDNAs for P2X7R and HA-v3 or -v4, followed by 48-h post-incubation in 10% FBS-EMEM were used (transient). In panels b and e, and c and f, Western blotting was performed using lysates, and membrane and cytosol fractions, respectively, of cells as samples. In panels c and f, cadherin and GAPDH are loading controls for the membrane

and cytosol fractions, respectively. Representative photomicrographs (a) and Western blots (b, c, e, f) for three independent experiments are shown, the quantitative results for panels c and f being given in panels d and g, respectively. Each column represents the mean±SD (N=3). *p<0.05 (*vs*. each mock). Bar = 20 μm.



sFigure 5. Expression of P2X7R in astrocytes is not altered by OGD-treatment.
After cells had been incubated under hypoxic conditions at 37°C for 2 h in a multi-gas incubator, they were cultured in 5.6 mM glucose-containing EBSS with oxygen for 24 h. Thereafter, the expression levels of P2X7Rs in cell lysates were determined by Western blotting. Representative images and the quantitative results for three independent experiments are shown. Each column represents the mean±SD (N=3). *p<0.05 (vs. each control).